CLAIMS

We claim:

1. A method for producing producing therapeutic human T regulatory cells (Treg cells) with enhanced suppressive activity, said method comprising selecting a sample of $CD4^+$ T cells; and isolating from said sample a population of human $CD4^+CD25^+$ suppressor T cells, and *ex vivo,* long-term, culture-expanding the $CD4^+CD25^+$ cells by GMP-approved methods, thereby activating potent long-term suppressor activity in the isolated, culture-expanded cells, wherein prior to expansion the natural population of $CD4^+CD25^+$ suppressor cells represents a low percentage of the total isolated $CD4^+$ T cell population.

2. The method of claim 1, wherein isolation of the $CD4^+CD25^+$ cells comprises a high level of stringency.

3. The method of claim 2, wherein said isolation of the $CD4^+CD25^+$ cells further comprises purifying the isolate by substantially enhancing $CD4^+CD25^{bright}$ cells in the population, while substantially depleting $CD25^{dim}$ cells in the population.

4. The method of claim 3, wherein said purification method comprises contacting the isolate with conjugated anti-CD25 magnetic microbeads at a predetermined bead/cell ratio, and purifying by running the bead/cell composition over a magnetic column to separate bead-bound cells, washing, and re-eluting over a second magnetic column, and again washing until <1-2 % of nonsuppressor cells remain in the purified isolate.

5. The method of claim 1, wherein culture-expanding the $CD4^+CD25^+$ cells comprises activating the isolated cells by a second-generation lineage depletion protocol using two steps and a cleavable cell-sized, antibody-coated, magnetic microbeads, thereby amplifying the culture-expanded Treg suppressor cells over a sufficient period of time until there exists in the cell culture an effective amount of suppressor cells to achieve therapeutic suppression of an immune or autoimmune response in a human.

6. The method of claim 5, wherein the microbeads are coated with antibodies to CD3 and CD28, thereby augmenting activation and growth of hypoproliferative suppressor Treg cells.

7. The method of claim 6, further comprising supplementing media for culture-expanding the cells with IL-2.

8. The method of claim 6, further comprising achieving at least 10-20 fold expansion of the cells within 14 days of culture.

9. The method of claim 8, further comprising achieving at least 100-fold expansion of the cells by culturing the cells for an additional 1-2 weeks.

10. The method of claim 6, further comprising generating suppressor cell lines that retain log term down-regulatory suppressor function.

11. The method of claim 1, wherein the sample is selected from the group consisting of whole or partially purified blood or hematopoietic cells, selected from the group consisting of peripheral blood mononuclear cells, peripheral blood lymphocytes, spleen cells, tumor-infiltrating lymphocytes and lymph node cells, and bone marrow and peripheral bone marrow cells.

12. A population of activated and *ex vivo* culture-enhanced suppressor Treg cells produced by the method of claim 1, wherein suppressor function is retained long term and expanded cell number is sufficient for effective therapy in humans.

13. A population of activated and *ex vivo* culture-enhanced suppressor Treg cells produced by the method of claim 2, wherein the suppressor cells are first purified at a high level of stringency, and wherein suppressor function is retained long term and expanded cell number is sufficient for effective therapy in humans.

14. A population of activated and *ex vivo* culture-enhanced suppressor Treg cells produced by the method of claim 5, wherein suppressor function is retained long term and expanded cell number is sufficient for effective therapy in humans.

15. A population of activated and *ex vivo* culture-enhanced suppressor Treg cells produced by the method of claim 5, wherein the suppressor cells are first purified at a high level of stringency, and wherein suppressor function is retained long term and expanded cell number is sufficient for effective therapy in humans.

16. A population of activated and *ex vivo* culture-enhanced suppressor Treg cells produced by the method of claim 6, wherein suppressor function is retained long term and expanded cell number is sufficient for effective therapy in humans.

17. A method for inhibiting alloreactive T cell expansion and cytokine production comprising contacting said alloreactive T cells with activated long-term, *ex vivo* culture-expanded Treg cells produced by the method of claim 1.

18. A method for inhibiting CTL activity comprising contacting said cells with activated long-term, *ex vivo* culture-expanded Treg cells produced by the method of claim 1.

19. A method for achieving an immunosuppressive effect in a patient comprising administering to said patient with an alloresponse or autoimmune response, an effective amount of activated long-term, *ex vivo* culture-expanded Treg cells produced by the method of claim 1 to achieve therapeutic suppression of said response.

20. The method of claim 19, further comprising suppressing, blocking or inhibiting *in vivo* alloresponses or autoimmune responses in a patient comprising administering to said patient with said alloresponse or autoimmune response, an effective amount of activated long-term, *ex vivo* culture-expanded Treg cells.

21. The method of claim 19, wherein the patient's response follows tissue transplantation, and wherein the method further comprises suppressing, blocking or inhibiting graft-vs-host disease in the patient.

22. A method for achieving a preventative therapeutic effect in a patient comprising administering to said patient, prior to onset of an alloresponse or autoimmune response, an effective amount of activated long-term, *ex vivo* culture-expanded Treg cells produced by the method of claim 1 to prevent said response.

23. The method of claim 22, further comprising preventing *in vivo* alloresponses or autoimmune responses in a patient by administering to said patient prior to the onset of said response, an effective amount of activated long-term, *ex vivo* culture-expanded Treg cells.

24. The method of claim 22, wherein the patient is treated prior to, at the time of, or immediately after tissue transplantation, and wherein the method further comprises preventing onset of graft-vs-host disease in the patient.

25. The method of claim 22, wherein the patient is treated prior to, at the time of, or immediately after tissue transplantation, and wherein the method further comprises blocking rejection of the transplanted tissue in the patient.